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OVEREXPRESSION OF FRIZZLED RELATED PROTEIN INCREASES SYNOVITIS IN A MOUSE MODEL OF ARTHRITIS

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Purpose: Frizzled related protein (FRZB/sFRP3) is a secreted WNT antagonist isolated from articular cartilage and expressed in developing skeletal elements. Polymorphisms in the human *FRZB* gene are associated with susceptibility for osteoarthritis. Induction of experimental osteoarthritis in *Frzb*$^{-/-}$ mice results in enhanced cartilage degradation associated with increased Wnt signalling, *Mmp3* expression, Mmp activity and cortical bone thickness. We further studied the role of FRZB in osteoarthritis by *FRZB* adenoviral overexpression in the knee joint in the methylated bovine serum albumin (mBSA)-induced arthritis model.
Methods: Adenovirus expressing *FRZB* or *GFP* (10^7 pfu/10 μl in PBS) was injected intra-articularly in the right knee joint of C57Bl/6 mice at day 0. At day 1 mBSA-induced arthritis was induced by intra-articular injection of 10 μl mBSA (20 mg/ml in PBS) followed by subcutaneous injections of IL-1β (250 ng) in the right footpad for 3 consecutive days. The left knee was used as a negative control and injected each time with 10 μl of PBS. At day 7, mice were sacrificed and the knee, synovium or articular cartilage were isolated for histology or mRNA analysis. Knee sections were stained with haematoxylin-eosin and safranin O and severity of arthritis was scored. Expression of inflammatory cytokines *Il-1β*, *TNF-α* and *Il-6* and matrix-degrading enzymes *Mmp3*, *Mmp9*, *Mmp13*, *Adamts4* and *Adamts5* in synovium and articular cartilage was determined using quantitative RT-PCR.
Results: Histomorphological analysis of the knee showed a significant increase in the score of inflammatory parameters such as infiltration, exsudate formation and pannus formation when *FRZB* was overexpressed. Cartilage damage was not different compared to GFP controls. Increased synovitis was confirmed by gene-expression analysis of inflammatory cytokines. *Il-1β*, *TNF-α* and *Il-6* expression were increased in the synovium with *FRZB* overexpression. We also detected a decreased expression of *Mmp3* in cartilage when *FRZB* was overexpressed and an increase of *Adamts5* in synovium. *Mmp9*, *Mmp13* and *Adamts4* expression did not change in synovium and articular cartilage. The altered balance between *Adamts5* and *Mmp3* expression may explain the absence of changes in cartilage damage.
Conclusions: In this study we demonstrate that *FRZB* overexpression leads to increased inflammation in mBSA-induced arthritis in mice. The increased inflammatory response appears to counteract potential chondroprotective effects of *FRZB* overexpression. These data further highlight the complex biology of FRZB.

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STREAMING POTENTIAL-BASED ARTHROSCOPIC DEVICE CAN DETECT CHANGES IMMEDIATELY FOLLOWING LOCALIZED IMPACT IN AN EQUINE IMPACT MODEL OF OSTEOARTHRITIS

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Purpose: Early Post-traumatic Osteoarthritis (PTOA) can be asymptomatic but represents an opportunity for therapeutic intervention. Impact models are ideal for studying these strategies because the location and severity of impact can be controlled. Streaming potentials generated during cartilage compression are a sensitive measure of degeneration. An arthroscopic device (Arthro-BST*) permits non-destructive electromechanical measurements and computes a Streaming Potential Integral (SPI) that reflects cartilage structure and function.
The purpose of this study was to assess whether streaming potentials detect changes in articular cartilage immediately following Low and High impacts delivered using a custom-built impactor device.
Methods: 32 sites on a medial femoral condyle of a 2-year old horse were evaluated using the Arthro-BST by two independent users who made 3 measurements at each site. At 16 sites, Low or High impacts were delivered to the articular surface using a custom-built impactor device with a 6.5 mm diameter plane-ended tip with rounded edges. The remaining 16 sites served as non-impacted controls. Following impact, Arthro-BST measurements were repeated and India ink was applied to the joint surface. Full thickness cartilage disks were extracted for testing in unconfined compression geometry, using a Mach-1 Micromechanical Tester*. Each disk was subjected to five stress relaxation ramps of 2% strain. The fibril-network-reinforced biphasic model was fit to the data to obtain fibril modulus (Ef), matrix modulus (Em) and hydraulic permeability (k). Cartilage disks were then fixed in neutral buffered formalin and embedded in paraffin. Sections, 10 μm thick, were stained with Safranin-O/Fast Green. Paired t-tests were used to compare pre- and post-impact SPI and biomechanical parameters at impacted sites versus adjacent normal sites.
Results: Consistent Low impact at 15.0±1.4 MPa (n=6) and High impact at 41.3±5.3 MPa (n=8) were delivered. India ink application revealed surface cracking and diffuse staining at High impact sites, and faint or no staining at Low impact sites.
A decrease in SPI at all sites that received a High impact was observed, although not statistically significant (p=0.09, n=8). There was no change in SPI following Low impact (p=0.65, n=6).
High impact samples had reduced Ef, representing collagen network stiffness (p=0.04), increased k (p=0.03) but no change in Em, representing proteoglycan matrix stiffness (p=0.21). This suggests that damage to the collagen network was immediate, but that there was insufficient time for substantial proteoglycan loss to occur. No changes in biomechanical properties were detected in the Low impact group.
Safranin-O/Fast Green staining showed some proteoglycan depletion at cartilage disk edges and the articular surface in both Low and High impact samples, possibly reflecting loss during the fixation period where no specific cationic agents were added to retain proteoglycan (Fig. 1).

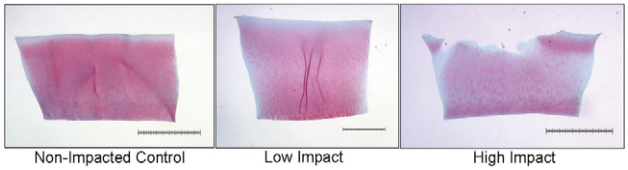

Figure 1. Representative images of Safranin-O/Fast Green stained sections from a non-impacted control site and sites that received either Low or High impacts. Length of the scale bar is 1 mm.

Conclusions: Changes in biomechanical properties and streaming potentials were detected after impact injury and could be used to study early degeneration. The non-destructive nature of the streaming potential method will permit sequential assessment of cartilage function over time for in vivo studies, where initial degeneration is focal but could progress to gradually involve more of the articular surface. Therapeutic agents to mitigate progression could be evaluated in this type of model.